

## Acquisition of sperm motility and its maintenance during storage in the lizard, *Lacerta vivipara*

A. Depeiges\* and J. L. Dacheux†‡

\**Biologie Cellulaire et Génétique, Université de Clermont-Ferrand II, BP. 45, 63170 Aubière, and*  
†*Laboratoire Physiologie Comparée, Faculté des Sciences, 37200 Tours, France*

**Summary.** Lizard spermatozoa, which are non-motile in the testis, develop the ability to swim as they pass along the excurrent duct. The addition of caffeine, a phosphodiesterase inhibitor, induced forward motility in spermatozoa from the caput epididymidis and increased the velocity of spermatozoa from the distal part of the epididymis. Caffeine had no effect on the motility of testicular spermatozoa. This suggests that sperm motility in this species is cyclic AMP-dependent but this factor alone is not sufficient to induce testicular sperm motility. In samples from the distal region of the epididymis, sperm motility was maximal in April just after the breeding season and then decreased significantly during the following months. A parallel can be drawn between these data and the levels of testosterone in the plasma. In the lizard, as in mammals, the epididymis may play an important role in the maturation of spermatozoa.

### Introduction

The spermatozoa of mammals of several species undergo changes during epididymal transit which result in the ability to fertilize ova (see review by Dacheux & Paquignon, 1980). One of the most obvious changes is the development of the ability to swim when the gametes are diluted in particular media. In mammals, the capacity for vigorous forward progression appears when the gametes are present in the distal part of the epididymis. Maturation of the spermatozoa of eutherian mammals depends on a special environment created by androgen-dependent activities in the epithelium of the epididymis.

The existence of subcellular changes in spermatozoa in the excurrent duct and the influence of this excurrent duct on the maturation of the spermatozoa of other vertebrates are little understood. Among the little information available (see review of Bedford, 1979) there are great variations between class and species. In lower vertebrates such as teleost fish, cyclostomes and frogs, spermatozoa released from the testis are motile and fully fertile, but modifications appear to have taken place in some species of birds and reptiles. In reptiles such as the lizard (*Lacerta vivipara*) (Gigon-Depeiges & Dufaure, 1977; Depeiges, Betail & Dufaure, 1981), the epithelium of the excurrent duct produces, under the control of testosterone during the breeding season, large secretory granules which are discharged and mixed with the spermatozoa during the reproductive period. At least one of the major proteins secreted is a sperm coating-protein (Depeiges & Dufaure, 1983).

The purpose of the present studies was to determine whether the spermatozoa of the lizard undergo a maturation process during epididymal transit and at different steps of the reproductive period. The motility of the spermatozoa has been used as an index of this postulated maturation.

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‡ Present address: Physiologie de la Reproduction, INRA, 37380 Nouzilly, France.

## Materials and Methods

**Animals.** Animals were captured in the Massif-Central (France) during their reproductive season between April and May. The first matings are generally observed during the second part of April. In this study the animals (4 in each group) were killed at the end of April, the middle of May and the end of May.

**Sperm samples.** The spermatozoa were collected from the testis and three sections of the excurrent duct. For the epididymis, the sections were defined on the basis of the histological analysis (Plate 1). The proximal region, the anterior part of the organ until the level of the inter-kidney gland, is characterized by epithelial cells which secrete numerous large granules. Very few spermatozoa are present in this part of the organ. The middle region begins at the adrenal gland and includes approximately the second third of the epididymis. In this part, the epithelium is taller and the lumen of the tubule is larger than in the first part. In the lumen, the concentration of spermatozoa is high and they are mixed with abundant secreted granules. The distal region of the organ is characterized by a white colour because it is packed with spermatozoa. The epithelial cells are lower than in the other areas and intracellular secretory granules are less abundant.

**Statistical analysis.** Significant differences between results were assessed by analysis of variance. Partitioning of treatment sums of squares was carried out by using a matrix of orthogonal polynomial coefficients.

**Diluents.** As in some mammals, the spermatozoa in their own epididymal medium are immotile. Motility can be induced after dilution in specific medium. In preliminary experiments, several diluents were tested: Krebs, MEM, Ringer, Tyrode buffer, and a medium adapted for birds (Lake & Ravie, 1979). The presence of protein (bovine serum albumin or egg yolk) in these diluents has also been tested. The medium which gave the most reproducible results was the Tyrode buffer (136 mM-NaCl; 2.6 mM-KCl; 1.8 mM-CaCl<sub>2</sub>, 1 mM-MgCl<sub>2</sub>, 12 mM-NaHCO<sub>3</sub>, 0.4 mM-NaH<sub>2</sub>PO<sub>4</sub>, 5.5 mM-glucose) with 5% egg yolk. In experiments in which phosphodiesterase inhibitor was used, 6 mM-caffeine was added to the Tyrode buffer.

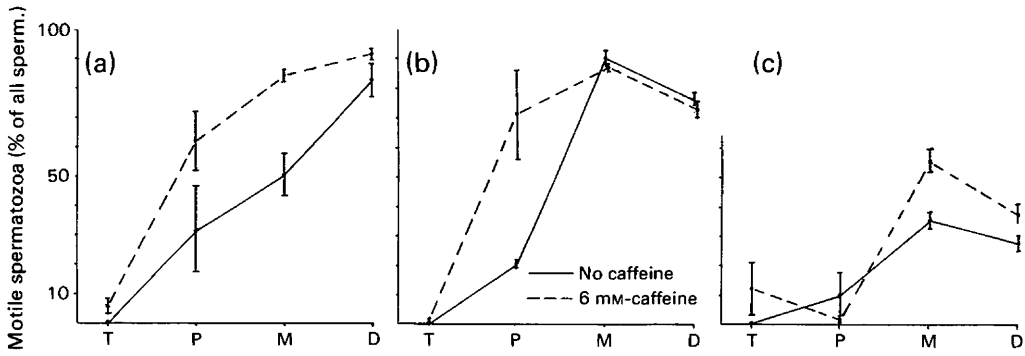
**Sample preparation and motility evaluation.** The genital tracts were dissected from animals killed by decapitation, spermatozoa were released by mincing the testis and the different areas of the epididymal tubule with scissors in Tyrode/egg-yolk solution, and incubated at 30°C, the optimum ambient temperature for the lizard. The motility of the spermatozoa was assessed after incubation for 10, 30 and 90 min. The motility of the spermatozoa was obtained by a photographic tracking method. A sample of sperm suspension (10 µl) was placed between a prewarmed coverslide (20 × 20 mm) and a slide. Photographs were taken with Kodak Tri-X film at an exposure time of 4 sec under 10 × 10 dark-field illumination. Percentages of motile spermatozoa and velocities were measured by examination of the tracks (at least 60 for each sample) on photographs placed on a digitalized table linked to a computer. In some experiments the progressive forward motility of the spermatozoa was recorded with a 16-mm film under phase-contrast light.

## Results

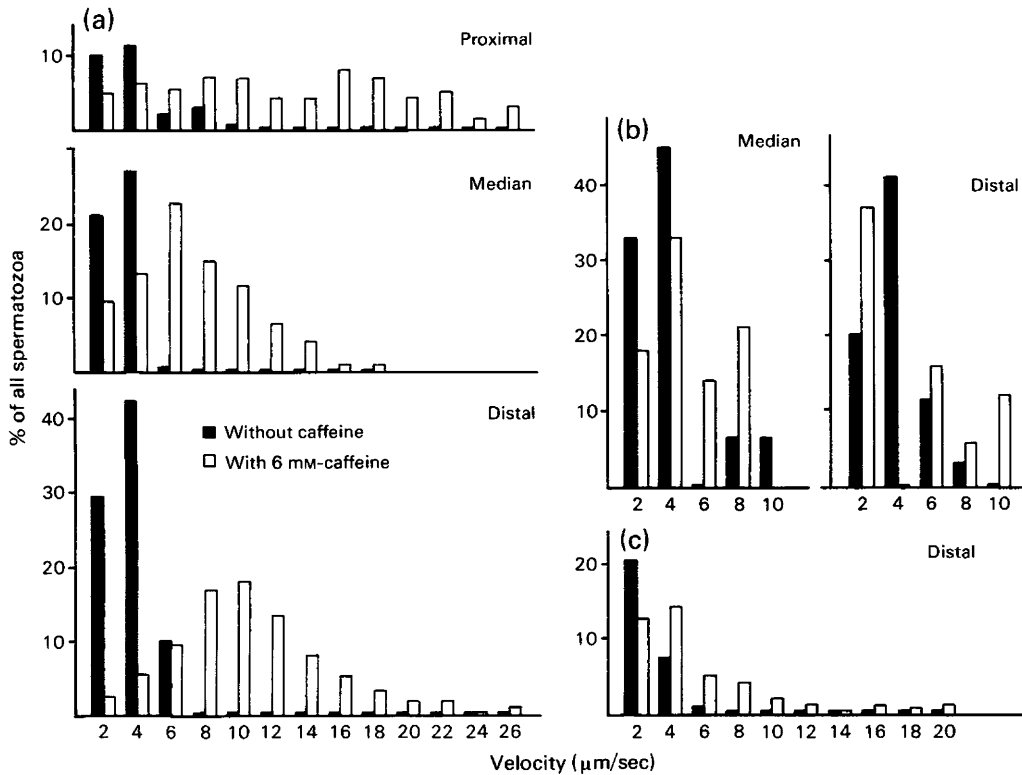
### *Sperm motility during the breeding period*

**Motility gradient in the genital tract.** The percentage of motile spermatozoa in the testis preparation was very low. Most of the spermatozoa were still linked to the Sertoli cells. In less than 1% of these spermatozoa or those that were free in the preparation, the flagella showed only a little irregular beating and none of the gametes was motile (Text-fig. 1a).

The percentage of motile spermatozoa increased as they passed through the epididymal tubule. In the proximal part of the epididymis, 31.5% of the spermatozoa had progressive motility (Text-



**Text-fig. 1.** Percentages of motile spermatozoa in different parts of the genital tract of lizards (T, testis; P, proximal; M, median and D, distal part of the epididymis) and in the presence or absence of caffeine during (a) the breeding period, (b) 15 days after the breeding period and (c) 30 days after the breeding period.



**Text-fig. 2.** Histograms of sperm velocity in the presence or absence of caffeine in successive segments of the epididymis (P, proximal; M, median; D, distal) of the lizard during (a) the breeding period, (b) 15 days after the breeding period, and (c) 30 days after the breeding period.

fig. 1a) with a velocity ranging from 2 to 6  $\mu\text{m}/\text{sec}$  (Text-fig. 2). The percentages of motile spermatozoa in the middle and distal regions were 50.2 and 82.2% respectively. Their velocities were the same as those in the anterior region (Text-fig. 2).

The progressively motile spermatozoa displayed helicoidal beating. For nearly all the spermatozoa observed, the forward progression was not uniform. Periodically the spermatozoa

stopped and a quiescent phase was observed for about 1 sec (Plate 2). During this phase, the flagella of the spermatozoa had a very characteristic shape: the flagellar beat stopped, and the flagellum stayed still (Pl. 2, Figs 10–17). At the same time, a sharp bend appeared in the flagellum near the head causing a retroversion of the head (Pl. 2, Figs 11–17). For 0.1–0.2 sec, the spermatozoa were completely immotile. After this phase, the head came back to its normal position and the propagation of the flagellar wave reappeared (Pl. 2, Figs 18–31).

*Effects of caffeine on sperm motility.* The presence of 6 mM-caffeine in the preparation of testicular spermatozoa increased the percentage of motile spermatozoa (0.5 to 7%, Text-fig. 1); a progressive motility of the spermatozoa seemed to develop but was too weak to be measured under the conditions of the experiment. Variations amongst animals were also observed.

For all spermatozoa collected in the epididymis, the percentage of motile spermatozoa increased significantly when the phosphodiesterase inhibitor was present in the medium (Text-fig. 1). The velocity of the gametes was also enhanced (Text-fig. 2a). These increases in velocity were greater for spermatozoa collected in the distal region of the epididymis than for those from the middle region (Text-fig. 2a). For spermatozoa from these two regions, the frequency distribution of the velocity was Gaussian-like (Text-fig. 2a) but for spermatozoa collected from the anterior region of the epididymis only 5–7% of spermatozoa were motile at the various velocities shown.

#### *Changes of motility after the reproductive period*

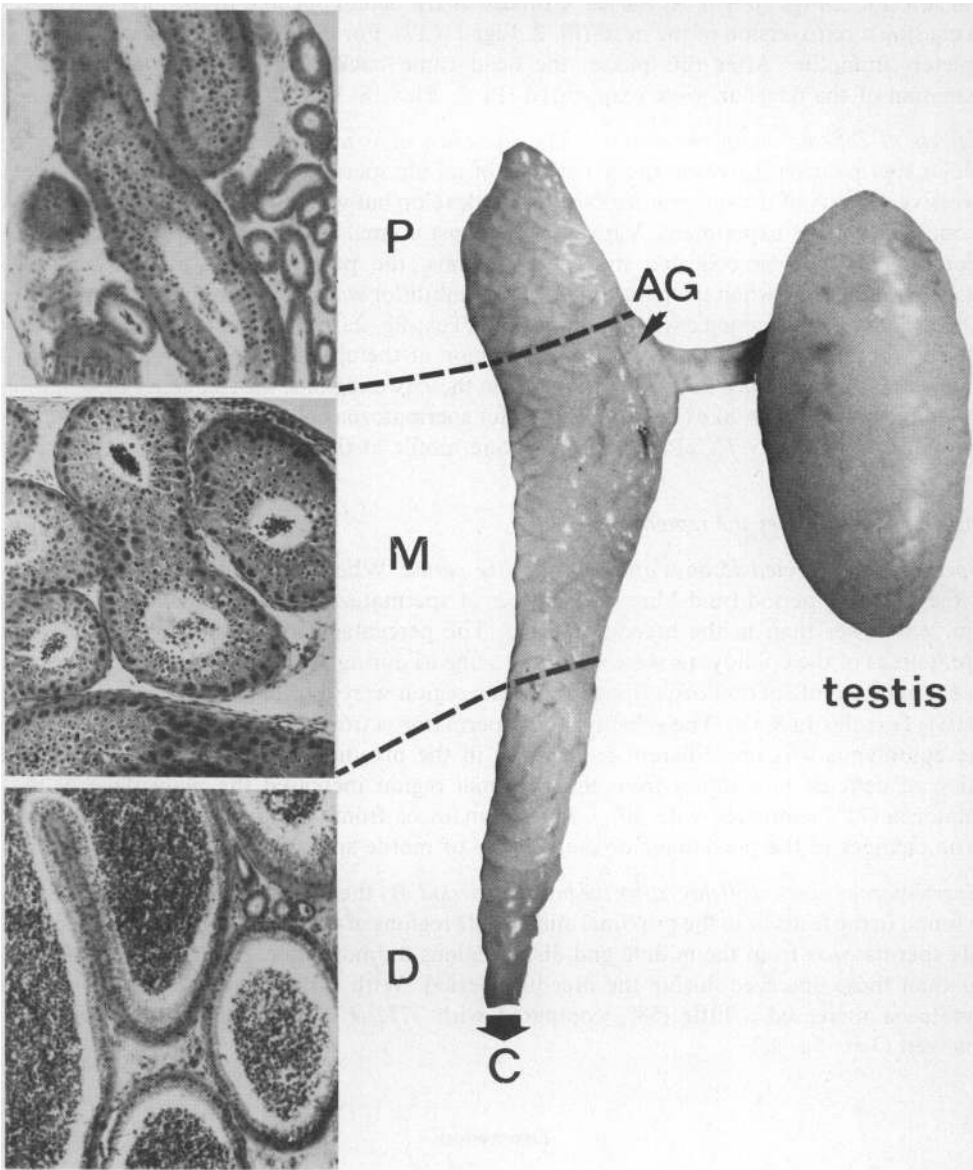
*Spermatozoa collected 15 days after the breeding period.* When the animals were killed 15 days after the breeding period (mid-May) the number of spermatozoa, in the testis and the proximal region, was lower than in the breeding period. The percentages of motile spermatozoa in the different areas of the epididymis were about the same as during the breeding period except that a higher proportion of spermatozoa from the middle region were motile (90% compared with 50%,  $P < 0.05$ ) (Text-figs 1a & 1b). The velocity of the spermatozoa from the middle and the distal region of the epididymis was not different from those in the breeding period (Text-fig. 2b), but the addition of caffeine to samples from the proximal region increased the percentage of motile spermatozoa (72% compared with 20%). For spermatozoa from the other parts of the duct there were no changes in the percentage or the velocity of motile spermatozoa (Text-figs 1 & 2b).

*Spermatozoa collected 30 days after the breeding period.* By the end of May, very few spermatozoa were found in the testis or in the proximal and middle regions of the epididymis. The percentages of motile spermatozoa from the middle and distal regions did not differ. However, the values were lower than those observed during the breeding period. With caffeine, the percentage of motile spermatozoa increased a little (58% compared with 37%,  $P < 0.05$ ), but their velocities were unchanged (Text-fig. 2c).

## Discussion

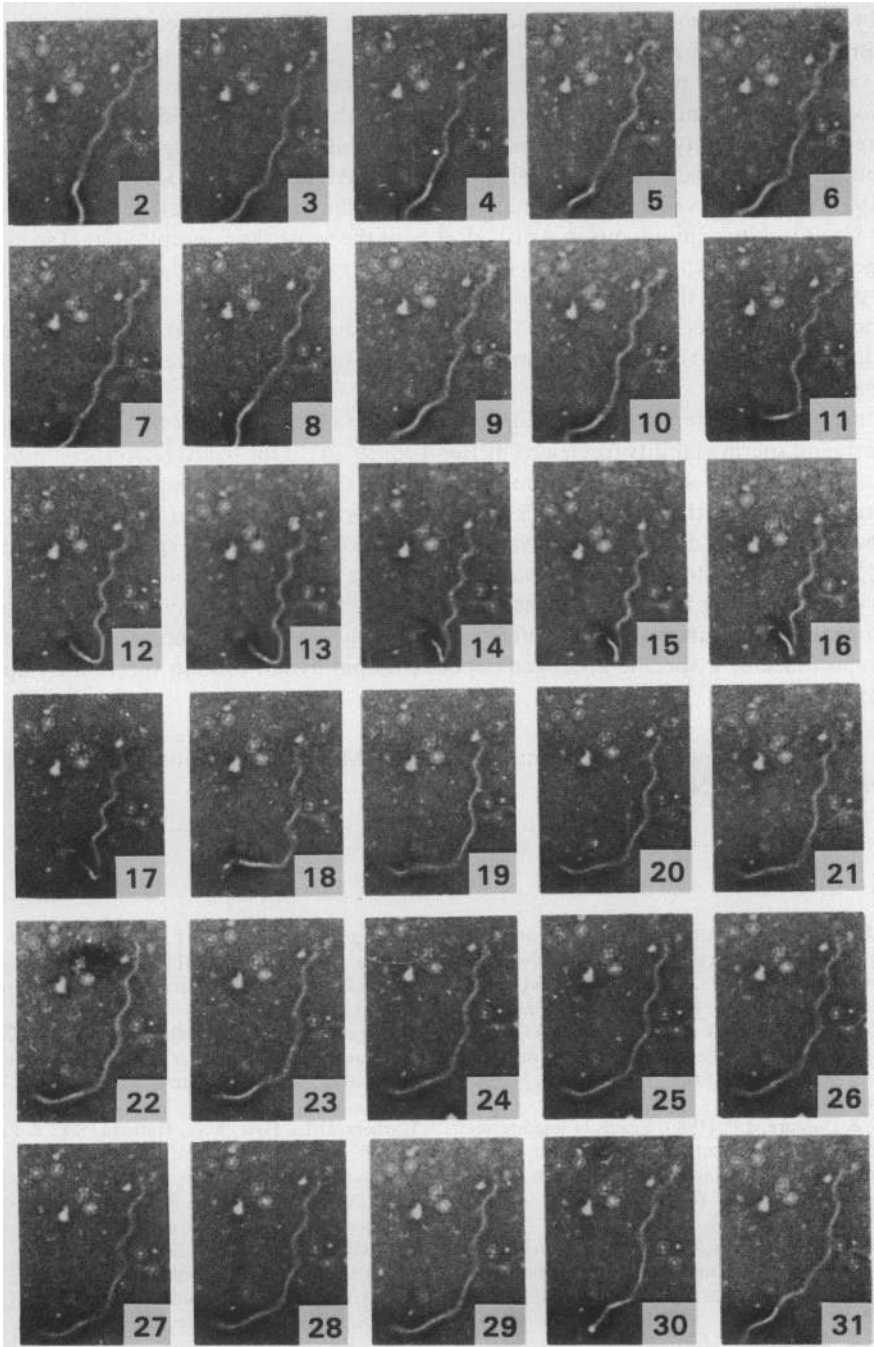
In the lower vertebrates there is generally a coincidence of internal fertilization with the appearance of some post-testicular maturation. In the work presented here, a maturation process has been demonstrated for the epididymal spermatozoa in the lizard. As described for mammals, the spermatozoa are immotile in the testis and develop the ability to swim as they pass along the epididymis.

Maximum percentages of motile spermatozoa and sperm velocities are reached in the distal segment where the spermatozoa accumulate. Progress in the medium by the motile spermatozoa is relatively slow. Although the intermittent swimming of the spermatozoa is unusual, a similar phenomenon has been described for sea urchin spermatozoa when they are illuminated with blue light (Gibbons, 1980). The origins of the characteristic quiescent phase are unknown. In a



**Fig. 1.** Anatomical and histological aspects of the lizard epididymis. AG = adrenal gland; C = cloaca; P, M, D = proximal, median and distal zones. Histological pictures are  $\times 112$ .

PLATE 2



**Figs 2–31.** Typical movement of lizard spermatozoa recorded by microcinematography at 24 frames/sec. The sequence of the images is from 2 to 31.

demembrated sperm model, calcium seemed to play a role in the appearance of this quiescent phase (Brokaw, Josselin & Bobrow, 1974).

The addition of a phosphodiesterase inhibitor (caffeine) had no effect upon testicular spermatozoa but it did induce some forward motility in the caput and middle region spermatozoa and increased the velocity of spermatozoa from the middle and distal regions. This suggests that sperm motility in this species, as in mammals, is cyclic AMP-dependent (Garbers, First, Gorman & Lardy, 1971). The origins of the epididymal influence on the sperm maturation are not clear but the spermatozoa reach the epididymis in April, after or at the same time, as epididymal secretions are discharged into the lumen. The percentages of motile spermatozoa are reduced when this secretory activity decreases after the breeding period.

Amongst the proteins synthesized by the epididymis during this period a major soluble protein, protein L (18 000 mol. wt) that binds to spermatozoa has been characterized (Depeiges & Dufaure, 1981, 1983).

In other studies on reptiles no relationship has been observed between testicular androgens, epididymis and sperm viability (reviewed by Bedford, 1979). In the lizard (*Lacerta vivipara*), sperm motility in the distal segment was maximal (80% of motility) when plasma testosterone concentrations were at the highest levels (400 ng/ml) for the year (Courty & Dufaure, 1980) and decreased progressively during the following month. By early June motile spermatozoa represented only 30% of all spermatozoa and plasma testosterone levels had fallen to 20 ng/ml.

This viviparous lizard therefore seems to be a useful animal for study of the testicular androgen involvement in the maturation and maintenance of viability of mature spermatozoa, and especially of the importance of a specific androgen-dependent epididymal protein (protein L) upon these phenomena.

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